Ultrastructural description of *Dientamoeba fragilis* and a new viral-like particle

By

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Certificate of original authorship

This study was conducted in the School of Medical and Molecular Biosciences and i3 institute, Faculty of Science, University of Technology, Sydney and in the Microbiology Department, St. Vincent's Hospital Sydney, under the supervision of Professor John T. Ellis and Dr. Damien Stark.

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me with editorial help from Prof. John Ellis and Dr. Damien Stark as acknowledged in individual chapters. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. Finally, I certify that all information sources and literatures used are indicated in the thesis.

Gouri Rani Banik
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Refereed publications arising from this thesis

1. Barratt, J.L., **Banik, G. R.**, Harkness, J., Marriott, D., Ellis, J.T and Stark, D., 2010. Newly defined conditions for the *in vitro* cultivation and cryopreservation of *Dientamoeba fragilis*: new techniques set to fast track molecular studies on this organism. *Parasitology*, 137: 1867-1878.
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2. **Banik, G. R.**, Barratt. J. L.N., Marriott, D., Harkness, J., Ellis, J. T., and Stark, D., 2011. A case-controlled study of *Dientamoeba fragilis* infections in children, *Parasitology*, 138: 819-823. http://www.ncbi.nlm.nih.gov/pubmed/21524324

3. Banik, G. R., Birch , D., Stark, D., Ellis, J. T., 2012. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: An emerging cause of human enteric disease. *International Journal for Parasitology*, 42: 139-153. http://www.sciencedirect.com/science/article/pii/S0020751911002785

4. **Banik, G. R.**, Birch, D., Stark, D., Ellis, J. T., 2013. Virus-like particles (VLPs) in *Dientamoeba fragilis*: an ultrastructural study (Submitted for publication in the *Journal of Parasitology*, November 2013).

Conference proceedings

■ **Banik, G. R.**, Barratt. J. L.N., Marriott, D., Harkness, J., Ellis, J. T., and Stark, D. A case-controlled study of *Dientamoeba fragilis* infections in children, **Poster presentation,** ICOPA, Melbourne, Australia, 15 -19th August, 2010.

■ **Banik, G. R.**, Barratt. J. L.N., Marriott, D., Harkness, J., Ellis, J. T., and Stark, D. A case-controlled study of *Dientamoeba fragilis* infections in children**, Poster presentation**, 27th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 9-10th November, 2010.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: An emerging cause of human enteric diseases**. Poster presentation**, 28th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 1-2nd November, 2011.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus life cycle. **Oral presentation**, Australian Society for Parasitology Annual Conference, Launceston, Tasmania, 2-5th July, 2012.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Poster presentation**, 29th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 20 -21st November, 2012.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Oral** and **Poster presentation,** Gordon Research Seminar and Conference on Physical Virology, Ventura, California, USA, 19-25th January, 2013.

■ **Banik, G. R.**, Birch, D., Stark, D., and Ellis, J. T. Electron microscopy characterisation of *Dientamoeba fragilis* virus-like particles, **Poster presentation**, New Horizons 2013, 30th Combined Health Science Conference, Kolling Building, Royal North Shore Hospital, NSW, 18 -20th November, 2013.

Abbreviations

Terms:

Ax	Axostyle
ATCC	American Type Culture Collection
BB	Basal Body
cDNA	Complementary Deoxyribonucleic Acid
Ch	Chromatin Bodies
Co	Costa
CP	Capsid Protein
CsCl	Caesium Chloride
DAPI	4', 6-diamidino-2-phenylindole
DFV	*Dientamoeba fragilis* Virus
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
DIC	Differential Interference Contrast
dsRNA	Double Stranded Ribonucleic Acid
Dv	Digestive Vacuole
ED	Electron Dense
EDTA	Ethylenediaminetetraacetic Acid
EGTA	Ethylene Glycol Tetraacetic Acid
EM	Electron Microscopy
ENV	*Eimeria necatrix* Virus
ER	Endoplasmic Reticulum
ESV	*Eimeria stiedae* Virus
EtOH	Ethyl Alcohol

Gc	Golgi Complex
GFP	Green Fluorescent Protein
GLV	*Giardia lamblia* Virus
HCl	Hydrochloric Acid
HIV	Human Immunodeficiency Virus
ITS	Internal Transcribed Spacer
LRV	*Leishmania* RNA virus
$MgCl_2$	Magnesium Chloride
My	Myelin Sheath
Mt	Microtubules
MTOC	Microtubule Organizing Center
Nm	Nuclear Membrane
NaCl	Sodium Chloride
Np	Nuclear Pore
ORF	Open Reading Frame
OsO4	Osmium Tetroxide
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
Pf	Parabasal Filament
Pm	Plasmalemma
Ps	Pseudopodia
PVA	Polyvinyl Alcohol
RNA	Ribonucleic Acid
RNase	Ribonuclease

RdRp	RNA Dependent RNA Polymerase
Rs	Rice Starch
RT-PCR	Real-time Polymerase Chain Reaction
ssRNA	Single Stranded Ribonucleic Acid
SAF	Sodium Acetate -Acetic Acid- Formalin
ScV	*Saccharomyces cerevisiae* Virus (ScV)
S.D.	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
ssRNA	Single-Stranded Ribonucleic Acid
SSU rRNA	Small Subunit Ribosomal RNA
sv	Small Vacuole
TBE	Tris Borate EDTA
TE	Tris-EDTA
TEM	Transmission Electron Microscopy
TM	Tris- $MgCl_2$
tRNA	Transfer RNA
TVV	*Trichomonas vaginalis* Virus
UTR	Untranslated Region
VLP	Virus-Like Particle
WSSV	White Spot Shrimp Viruses

Units:

°C	Degree Celsius
g	Relative Centrifugal Force

h	Hour
Kb	Kilobase
KDa	Kilo Daltons
Kg	kilogram
M	Molar
Mb	Megabase
µM	Micromolar
µm	Micrometre
µg	Microgram
µL	Microlitre
mg	Milligram
mL	Millilitre
mM	Millimolar
min	Minute
ng	Nanogram
nm	Nanometer
U	Unit

Abstract

Dientamoeba fragilis is a trichomonad protozoan found in the gastrointestinal tract of humans and is implicated as a cause of diarrhoeal disease. Despite its widespread occurrence and associated symptoms, very little is known about the biology and pathogenicity of *D. fragilis*. Advances in cell biology of other trichomonads means there is a need to advance knowledge on this neglected protozoan.

In this study, the morphological characteristics and ultrastructure of *D. fragilis* were described in detail using different microscopy techniques. Scanning electron microscopy, transmission electron microscopy, confocal and light microscopy were used to characterise *D. fragilis* populations growing in xenic culture. Under the SEM, two types of *D. fragilis* populations were identified based on cell surface structure: smooth cells and ruffled cells. Typically *D. fragilis* has a spherical or oval shape with a granular, vacuolated cytoplasm and some cells are amoeboid. *Dientamoeba fragilis* exhibited different motile forms with visible pseudopodia. The organelles in *D. fragilis* were analysed by transmission electron microscopy; the pelta, flagella, undulating membrane or pseudocyst-like forms were not found. The presence of hydrogenosomes in *D. fragilis* is described which has not been previously reported. The majority of cells grown in culture were mononucleate while most cells in permanent stained faecal smears were binucleate. Evidence is presented using confocal microscopy that the two nuclei of *D. fragilis* are identical in DNA content. In addition, the discovery of a virus-like particle is reported for the first time in *D. fragilis*. This study provides extensive and new detail on the ultrastructure of *D. fragilis* that is an emerging cause of human enteric disease.

Dientamoeba fragilis virus (virus-like particles or VLPs) was studied further: it was approximately 33-40 nm in size and the most common shape was spherical. These VLPs contain an inner dark core surrounded by an electron-lucent layer and an electron dense capsid coat. Virus particles are found extensively in the perinuclear region of the trophozoite, and especially around microtubules and in association with the Golgi complex. Virus particles were also found in the vicinity of endoplasmic reticulum, axostyle, and near to the parabasal filament but no VLPs were found in the nucleus.

Dientamoeba fragilis VLPs were also detectable in dying trophozoites present in *in vitro* cultures. Whether viral load contributes to cell death is not yet known.

The identity of the *D. fragilis* viral genome was also studied. Several different extraction methods were screened and three different methods were optimized to identify dsRNA from *Trichomonas vaginalis* (B7268 isolate) which was used as a positive control for the isolation of viral dsRNA. These optimized methods were evaluated to identify *D. fragilis* viral genome. No viral RNA or dsRNA was isolated from *D. fragilis* suggesting that unlike *T. vaginalis*, *D. fragilis* trophozoites do not contain a dsRNA virus, or that the abundance of the virus was so low that it prevented the identification of viral nucleic acid.

The epidemiology of *D. fragilis* has not been studied in detail and as a small side project I investigated hospital records for infections of children. Consequently, a case-controlled study was conducted to document the extent of *D. fragilis* infections in children presenting to a major Sydney Hospital. Treatment options are also discussed. In total, hospital data from 41 children were included in the study along with a control group. Results showed that diarrhoea (71%) was found to be the most common symptom followed by abdominal pain (29%). In addition, diarrhoea was statistically more significant in children with *D. fragilis* infection compared to a control group. In this study, the most common antimicrobial used for treatment was metronidazole (n=41), with complete resolution of symptoms and clearance of parasite occurring in 85% of cases. Moreover, a treatment failure rate of 15% was identified in children treated with metronidazole. These studies further suggest the pathogenic nature of *D. fragilis* and it is recommended that all laboratories must routinely test for *D. fragilis* as treatment which eliminates the parasite usually results in the resolution of symptoms.

In summary, this thesis has discussed many novel aspects on the biology of *D. fragilis* and provide new knowledge on the cell biology of this protozoan and a new protozoan virus.